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Aqueous Extract of *Sorindeia Juglandifolia* Leaves Protects Methotrexate-Induced Liver and Kidney Damage in Rat

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Abstract **Introduction**: Liver and kidney affection is a life-threatening disease caused by factors including drug-based treatment. Treatment based on methotrexate could result in liver and kidney damages. The study evaluates the preventive effects of *Sorindeia juglandifolia* leaves on methotrexate-induced liver and kidney impairment in rat. **Methods**: Healthy rats divided into 6 groups daily received distilled water, methotrexate (20 mg/kg), sub-cutaneous injection of L-carnitin (500 mg/kg) and methotrexate and the plant extract doses of 150, 250 and 350 mg/kg and methotrexate for 10 days. During treatment, body weight was recorded. At the end of the treatment, animals were sacrificed; venous blood were collected for haematological and biochemical analysis. Liver and kidney were collected for oxidative markers and histological examination. **Results**: The consecutive treatment of animals with plant extract and methotrexate showed a significant prevention of the body weight decrease and enhancement of the relative weight of liver and kidney. *Sorindeia. juglandifolia* extract also protected from the significant increase in transaminase activities, bilirubin and protein level, hypercholesterolemia, atherogenic index, and in the kidney from hypercreatininemia and the increase in serum urea level. The extract prevented the decrease of sodium level and glomerular filtration. Plant extract improved reactive oxygen species detoxification agents and protected from the histological disorganization of the liver and kidney tissues, observed in the MTX control. **Conclusion**: *Sorindeia juglandifolia* leaves extract expressed hepatorenal protective properties and could be useful to prevent liver and kidney damage induce by methotrexate.

Keywords: *Sorindeia juglandifolia*, hepatorenal affection, methotrexate, preventive activities

1. Introduction

The liver and kidney affections are prevalent diseases affecting about 2 -7% people worldwide. An estimate of 40000 annual deaths could be due to liver failure [1] while kidney damage affects about 7.2% of people aged of about 30 years old and 35.8% in individuals aged of more than 65 years over the world [2]. The physiological connectivity between liver and kidney is responsible for the activity of one to another. Thus, the toxicological susceptibility of liver and kidney is related to their physiological activities through biotransformation and excretion activities against xenobiotics [3]. The liver is implicated into biotransformation of xenobiotic products and the kidney ensures of their elimination. Indeed, the renal failure results in a reduction in clearance and tubular secretion leading to an increase of waste in the organism, responsible for exhaustion in the liver system. Likewise, the dysfunction of liver result in the detoxification failure that provoke a poisons accumulation in the organism and cause cellular damage especially in kidneys [4]. Hence, renal dysfunction is a common disease due either as part of multiorgan involvement in acute illness or secondary to advanced liver disease.

Furthermore, liver and kidneys are both implicated in the vitamin D production, blood pressure regulation through the renin-angiotensin system [5]. Liver and kidney affection could result from several factors including infectious diseases, toxins or drug exposure [6]. Twenty percent of kidney failure enamate from liver injury treatment [7] and 50 % of liver and kidneys damages are the results of anti-inflammatory, antibiotics and anti-cancer drugs as methotrexate [6,8,9]. Methotrexate (MTX) isa folic acid dihydrofolate reductase inhibitor used in the treatment of certain neoplastic, autoimmune and inflammatory diseases. A major side effects of its intake has highlighted the role of oxidative stress in causing toxicity on organs including the liver and kidney, due to the influx of methotrexate into the cell via active transport across the reduced folate carrier and its effluxes from the cell by ATP-binding cassette transporters [10,11]. An inhibition of both enzymatic and nonenzymatic antioxidants resulting in an increase of reactive oxygen species levels has been reported in the liver, kidney and other tissues of laboratory animals given methotrexate [13,14]. Toxic effect of MTX is related to a suppressive activity of folate metabolism include nodulosis, hepatic fibrosis, pulmonary fibrosis, lethargy, fatigue and renal failure [15]. Likewise, studies have reported the role of the adenosine pathway in methotrexate-induced hepatic fibrosis [16,17]**.** Some modern drugs have been developed to control these disturbances [6]. However, due to some side effects occurrence combined to the high cost of drugs, population in developing countries targeted medicinal plants to relieve their illness. Therefore, the urgent need to find out a hepatic-nephroprotective agent is mandatory for the safe use of this important drug. The use of plants to cure diseases is more frequent due to the efficiency and availability. *Sorindeia julandifolia* (Anacardiaceae) leaves are traditionally used for liver and kidney affection, but, no scientific evaluation has been done yet to demonstrate its activity. The aim of this study wasto evaluate the preventive effect of aqueous extract of *Sorindeia juglandifolia* leaves on methotrexate-induced liver and kidney damages in rat model.

2. Methodology

2.1. Extraction procedure and phytochemical analysis

Sorindeia juglandifolia (twigs with leaves) was harvested in Kala Mountain in Yaoundé-Cameroon in October2011. Plant authentication was done by Mr. Mizili, a botanist at National Herbarium Yaoundé, in comparison with another specimen deposited in National Herbarium under number 1976 SRF Cam. The leaves were collected, air dried and pulverized. Nine hundred grams (900 g) of powder were decocted in 9.0 L of distilled water for one hour following the instructions of tradipractitionner healers, and then filtered with Wattman paper n°3. The filtrate was freeze-dried giving a crude extract of 200 g with 22.22% of yield. Phytochemical analysis of extract was carried out using standard methodology described by Harborne, [18], Odebiyi & Sofowora, [19], Evans & Trease, [20], Sofowara, [21].

2.2. Experimental animals

Ten weeks old males *Wistar* rats weighing about 150 g were used for experimentation. The animals were housed in sanitary cages, at room temperature (22 ± 2 °C) on a 12 h light-dark natural cycle in the animal house of the Faculty of Science of University of Yaoundé I, Cameroon. Food and water were given *ad libitum* during the experiment. The study protocol has been approved by Institutional Ethical Committee, which adopted all procedures recommended by the European Union on the protection of animals used for scientific proposes (CEE Council 86/609; Ref N °FWA-IRD 0001954).

2.3. Study procedure

The study was carried following the protocol of Chauhan et al [22] with slightly modification. Six groups of 5 healthy animals each were treated for 10 days with methotrexate Bellona® as followed: A neutral control group received distilled water (10 mL/kg), the methotrexate (MTX) control, treated with methotrexate (20 mg/kg) and the L-carnitin control receiving by sub-cutaneous route,,L-carnitin (500 mg/kg) followed two hours later by oral methotrexate (20 mg/kg). Three test groups were treated with aqueous extract of *Sorindeia juglandifolia* at the dose of 150, 250, 350 mg/kg, then, by methotrexate (20 mg/kg), two hours later. The different treatments were administered for ten days. During experiment, body weight was daily recorded. At the end of experimentation, the animals were fasted for 12 hours, sacrificed under

anesthesia with intraperitoneal injection of a combination of ketamin (30 mg/kg) and diazepam (10 mg/kg). Blood samples were then, collected for hematological and for biochemical analysis. Liver and kidney were carefully removed, weighted and a section (2 g) of each organ was crushed with 2 mL of TRIS buffer, homogenate was collected for oxidative parameters analysis. The remaining portion were fixed into 10 % buffered formalin for histopathological examination.

2.4. Assessment of S. *juglandifolia* extract on biochemical analysis

All biochemical parameters were performed according to standard protocols using Immesco kits (Germany). Serum biochemistry parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, bilirubin level, lipid profile (total cholesterol, HDL and LDL cholesterol, triglycerides), and some anti-oxidative parameters (glutathione (GSH), Superoxyde dismutase (SOD), catalase, Malonedialdehyde (MDA) and nitrites).

2.5. Hematological parameters analysis

Ethylene diamine tetra acetyl acid (EDTA) blood samples were used for hematological parameters assessment. Red blood cells count (RBC), Hemoglobin (HGB), hematocrit (HCT), total platelets count, white blood cells (WBC), percentage of lymphocytes, monocytes and granulocyte were determined.

2.6. Histopathological analysis

The liver and kidney of each animal fixed in 10 % buffered formalin were dehydrated with gradual concentrations of alcohol and then embedded in paraffin. Serial paraffin sections at 5 µm were stained with hematoxylin and eosin (HE) for examination under light microscopy brand Olympus and photography in objective 40 auricular 100 (HEx400).

2.7. Statistical analysis

The results were expressed as mean ± standard error of mean (SEM).The statistical analyses of data were performed using the Analysis of Variance, followed by the post test of Benferroni through GraphpadInstat software 5.03 version. Significant difference was considered for $P < 0.05$.

3. Results

3.1. Qualitative screening of the plant extract

The aqueous extract of *Sorindeia juglandifolia* leaves revealed to contain metabolites such as polyoses, gallitannins, catecholamins tannins, phenolic component, saponins, anthraquinons, anthracenic, coumarins, fatty acid, alkaloids, flavonoids, terpenoides and sterols.

3.2. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on body weight and relative weight of organs

The effects of extract on the body weight evolution of animal are shown in the **Figure 1A.** It was noted that daily administration of MTX to healthy animal for ten days resulted in a significant decrease ($p < 0.001$) of body weight in Mtx control compared to the neutral control.The treatment with extract at the dose of 250 and 350 mg/kg significantly prevented the decrease ($p < 0.001$) of body weight in comparison with the Mtx control. It was observed significant decrease of body weight ($p < 0.001$) of animal receiving the extract dose of 150 mg/kg from the day 6 (D6) to day 10 (D10), compared as well as to the neutral control, L-Carnitin control and dose extract (250 and 350 mg/kg). At the end of treatment, no significant change was observed in body weight of animals treated with extract (250 and 350 mg/kg) and L-Carnitin.
MTX induced a significant increase in the relative weight of liver by 22.00% ($p < 0.05$) in MTX control compared to the neutral control (Figure 1B**)**. Plant extract administration (150 mg/kg) prior methotrexate led to a significant increase in

relative weight of liver by 17.63% ($p < 0.05$) compared to the neutral control. The relative weight of kidneys was significantly increased ($p < 0.05$) by 39.32 %at the extract dose of 150 mg/kg compared to the MTX control.

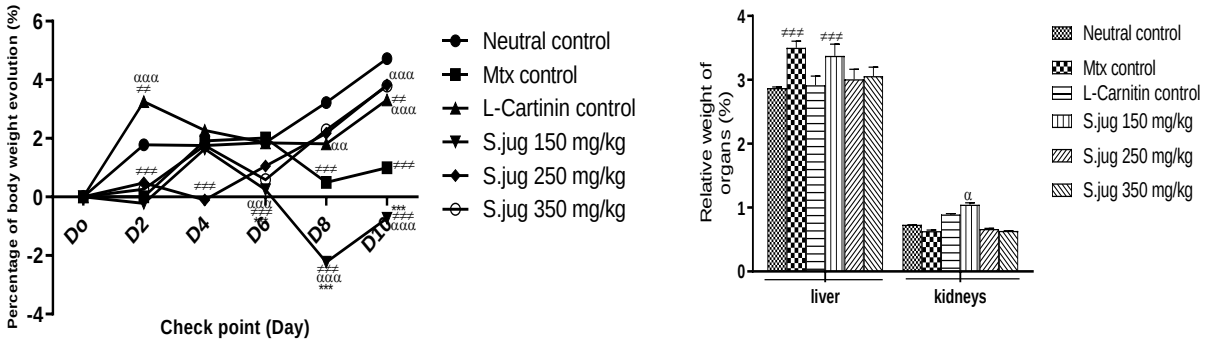

Figure 1. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on body weight evolution (A) and on the relative weight of liver and kidney (B).

Values are expressed as mean ± SEM (n= 5), Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration. $^{\neq}p < 0.05$, $^{\neq\neq}p < 0.01$, $^{\neq\neq\neq}p < 0.001$: significant difference compared to the neutral control, $^{\alpha}p< 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$: significant difference compared to the MTX control, *p < 0.05, **p < 0.01, ***p < 0.001: significant difference compared to the L-carnitin control.

3.3. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on some liver parameters.

The Table 1 presents the effects of aqueous extract of *Sorindeia juglandifolia* leaves in liver of animals treated with methotrexate for 10 days. It appears that, daily administration of methotrexate resulted in a significant increase in transaminase activities by 40.78% ($p < 0.05$) and 37.21% ($p < 0.01$) in ALT and AST respectively, in total bilirubin by 21.85 % ($p < 0.05$) and in serum protein levels by 30.18 % ($p < 0.01$) compared to the neutral control. A significant decrease in AST and ALT activities was observed in animal treated with the plant extract respectively by 42.60 % ($p < 0.01$) and 22.13 % ($p < 0.05$)at the dose of 150 mg/kg, by 37.97 % and 40.83% ($p < 0.01$), at 250 mg/kg and by 39.54 % and 43.51% ($p < 0.01$) at 350 mg/kg compared to the MTX control. The L-carnitin administration induced significant decrease of ALT and AST activity by 40.92 % ($p < 0.05$), and 40.01% ($p < 0.01$), respectively. The treatment with the plant extract at the dose of 150 mg/kg and 250 mg/kg followed methotrexate, provoked a decrease in total bilirubin level by 16.17% ($p < 0.05$) and 27.76 % ($p < 0.01$) respectively, compared to the MTX control. Serum proteins level increased ($p < 0.05$) by 27.56%, 29.61% and 27.65% at the respective dose extract of 250 mg/kg and 350 mg/kg and with L-carnitin (500 mg/kg). No significant change was observed in ALP activity in the experimental groups.

Table 1. Effects of aqueous extract of *Sorindeia juglandifolia* on some serum parameters of liver function

	ALT (UI/L)	AST (UI/L)	Bilirubin rate (mg/dL)	ALP (UI/L)	Serum proteins (mg/dL)
Neutral control	174.56 ±13.48	257.60 ±16.29	8.75 ±1.09	125.25 ±18.20	0.56 ±0.01
MTX control	294.77 ±14.95$^{\neq}$	410.29 ±51.61$^{\neq\neq}$	11.19 ±0.80$^{\neq}$	141.80 ±14.44	0.39 ±0.00$^{\neq\neq}$
L-carnitin control	176.82 ±23.92$^{\alpha}$	242.36 ±35.01 $^{\alpha\alpha}$	9.56 ±0.36	129.99 ±10.35	0.54 ±0.06$^{\alpha}$
S.jug 150 mg/kg	229.52 ±27.72$^{\alpha}$	235.47 ±34.22$^{\alpha\alpha}$	9.38 ±0.56$^{\alpha}$	127.00 ±4.25	0.49 ±0.01
S.jug 250 mg/kg	174.39 ±26.01$^{\alpha}$	254.46 ±13.75 $^{\alpha}$	8.08 ±0.21$^{\alpha\alpha}$	126.66 ±22.90	0.54 ±0.03$^{\alpha}$
S.jug 350 mg/kg	166.50 ±31.50$^{\alpha\alpha}$	248.05 ±21.33$^{\alpha\alpha}$	10.14 ±0.14	123.97 ±10.91	0.56 ±0.03$^{\alpha}$

Values are expressed as mean ± SEM, (n = 5). Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg)

and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration. ALT = alanine aminotransferase, AST = aspartate aminotransferase, APL = alkaline phosphate, $^{\neq}p < 0.05$, $^{\neq\neq}p < 0.01$: significant difference compared to the neutral control, $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$: significant difference compared to the MTX control.

3.4. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on lipid profile

The effects of aqueous extract of *Sorindeia juglandifolia* leaves on lipid profile are summarized in Table 2. Ten days administration of MTX led to a significant increase in total cholesterol by 26.71%, ($p < 0.05$), LDL-cholesterol by 56.38% ($p < 0.01$), atherogen index by 25.99% ($p < 0.05$), and a significant decrease in HDL-cholesterol by 38.84% ($p<0.001$) compared to the neutral control. In comparison to MTX control, the plant extract significantly prevented the increase($p < 0.05$) of the total cholesterol level by 21.18%, 29.02% and 35.45% , the LDL-cholesterol concentration by 38.02%, 51.65% ($p < 0.05$) and 63.21% ($p < 0.01$), atherogen index by 41.81%, 58.63% and 61,34 %($p < 0.001$) and the decrease of HDL-cholesterol level by 24.58%, 40.01% and 38.22 % ($p < 0.05$) at the respective dose of 150, 250 and 150 mg/kg. L-carnitin injection induced significant decrease of total cholesterol by 29.32% ($p < 0.05$), LDL-cholesterol by 53.88% ($p < 0.01$) and atherogen index by 58.15% ($p < 0.001$) and rather the decrease of HDL-cholesterol by 24.58% ($p < 0.05$). A significant decrease in HDL-cholesterol ($p < 0.05$) and atherogen index ($p < 0.05$) was observed in animal treated with extract (150 mg/kg) compared to L-carnitin control. No significant change was observed in triglycerides level in experimental groups.

Table 2. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on lipid profile

	Total Cholesterol (mg/dL)	Cholesterol LDL(mg/dL)	Cholesterol HDL (mg/dL)	Triglycerides (mg/dL)	Atherogen index
Neutral control	62.41 ±2.70	28.04 ±2.30	23.98 ±0.45	51.92 ±4.54	2.61 ±0.15
MTX control	85.16 ±2.32$^{\neq}$	55.99 ±3.77$^{\neq\neq}$	14.66 ±1.13$^{\neq}$	72.49 ±2.35	5.99 ±0.60$^{\neq\neq}$
L-carnitin control	60.19 ±6.46$^{\alpha}$	25.82 ±6.5$^{\alpha\alpha}$	24.12 ±0.50$^{\alpha}$	51.23 ±0.78	2.50 ±0.29$^{\alpha\alpha\alpha}$
S.jug 150 mg/kg	67.12 ±2.48$^{\alpha}$	34.70 ±2.42$^{\alpha}$	19.44 ±1.11$^{\neq\alpha*}$	64.87 ±9.11	3.48 ±0.19$^{\neq\alpha\alpha\alpha*}$
S.jug 250 mg/kg	60.44 ±2.43$^{\alpha}$	27.07 ±4.70$^{\alpha}$	24.44 ±0.33$^{\alpha}$	44.61 ±10.58	2.47 ±0.12$^{\alpha\alpha\alpha}$
S.jug 350 mg/kg	54.96 ±0.12$^{\alpha}$	20.60 ±1.38$^{\alpha\alpha}$	23.73 ±0,11$^{\alpha}$	53.16 ±6.88	2.31 ±0.00$^{\alpha\alpha\alpha}$

Values are expressed as mean ± SEM, (n= 5). Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration., $^{\neq}p < 0.5$, $^{\neq\neq}p < 0.01$: significant difference compared to the neutral control,$^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$: significant difference compared to the MTX control, $*p < 0.05$: difference significant compared to the L-carnitin control.

3.5. Effects of *Sorindeia juglandifolia* leaves extract on some serum parameters in kidney

Table 3 shows that daily administration of methotrexate for ten days provoked a significant increase in serum urea level (70.85%,$p < 0.01$)and creatinine (72.23 %, $p < 0.001$), whereas, a significant decrease in serum sodium (43.37 %,$p < 0.001$) and in the glomerular filtration volume (84.72%,$p< 0.001$)was observed compared to the neutral control. Compared to the MTX control, treatment with plant extract prior to methotrexate administration induced a significant decrease in serum urea and creatinine concentration by 57.88% ($p < 0.05$) and 56.47 % ($p < 0.01$) at the dose of 250 mg/kg and by 50.49% and 54.26% ($p < 0.05$) at the dose of 350 mg/kg, respectively. Treatment with L-carnitin has significantly decreased the serum urea and creatinine levels by 67.72 % and 56.79 % ($p < 0.01$) respectively. A significant increase in serum sodium concentration ($p < 0.001$) by 52.12%, 50.53%, 51.49 % and 51.91% was recorded with plant extract at the dose of 150 mg/kg, 250 mg/kg and 350 mg/kg and with L-carnitin respectively, compared to the methotrexate control. It was observed a significant increase in the glomerular filtration by 47.66% ($p < 0.001$) at the dose extract of 250 mg/kg and L-carnitin.The extract at the dose of 150 mg/kg failed to prevent the increase in urea and creatinin concentration

Table 3. Effect of aqueous extract of *Sorindeia juglandifolia* on some serum parameters in kidney

Experimental group	Urea (mg/dL)	Creatinin (mg/dL)	Calcium (mg/dL)	Sodium mg/L	Glomerular filtration (mL/24 h)
Neutral control	9.46 ±0.82	0.23 ±0.01	11.00 ± 0.24	301,81 ±16,83	3.10^{-2} ±0.00
MTX control	32.46 ±1.35$^{\neq\neq\neq}$	0.85 ±0.09$^{\neq\neq\neq}$	10.67 ±0.23	169,67 ±13,34$^{\neq\neq\neq}$	1.10^{-3} ±0.00$^{\neq\neq\neq}$
L-carnitin control	10.47 ±1.32$^{\alpha\alpha}$	0.36 ±0.02$^{\alpha\alpha}$	11.4 ±0.25	352,89 ±12,09$^{\alpha\alpha\alpha}$	2.10^{-2} ±0.00$^{\neq\alpha\alpha\alpha}$
S.jug 150 mg/kg	28.46 ±5.50$^{\neq\neq\neq *}$	0.58 ±0.02$^{\neq\neq\neq **}$	11.27 ±0.25	357,44 ±15,74$^{\alpha\alpha\alpha}$	1.10^{-2} ±0.00$^{\neq\neq\neq *}$
S.jug 250 mg/kg	13.67 ±0.82$^{\alpha}$	0.37 ±0.02$^{\alpha\alpha}$	10.91 ±0.22	346,01 ±9,10$^{\alpha\alpha\alpha}$	2.10^{-2} ±0.00$^{\neq\neq\neq\alpha\alpha}$
S.jug 350 mg/kg	16.07 ±0.82$^{\alpha}$	0.38 ±0.04$^{\alpha}$	10.68 ±0.24	337,38 ±25,52$^{\alpha\alpha\alpha}$	1.10^{-2} ±0.00$^{\neq\neq\neq\alpha}$

Values are expressed as mean ± SEM; (N = 5). Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration. $^{\neq}p < 0.5$, $^{\neq\neq}p < 0.01$, $^{\neq\neq\neq}p < 0.001$: significant difference compared to the neutral control, $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$: significant difference compared to the MTX control, $^{*}p < 0.05$, $^{**}p < 0.01$: difference significant compared to the L-carnitin control.

3.6. Effects of the aqueous extract of *Sorindeia juglandifolia* leaves on some oxidative parameters

Methotrexate administration to animals for ten days induced significant disturbance in oxidative stress parameters marked by a significant decrease in catalase activity by 27.41 %,($p < 0.01$) in kidney, in glutathione concentration by 75.19% ($p < 0.001$) and 88.43 %, in nitrites content by 22.61% and 24.88% ($p < 0.001$), in SOD activity by 20.95% and 17.37%, $p < 0.001$) and in tissue proteins ($p < 0.001$) by 52.09% and 71.84% in liver and kidney respectively, compared to neutral control (Table 4)**.** The methotrexate administration also provoked a significant increase in MDA concentration by 77.16% and 51.75% ($p < 0.001$) in liver and kidney respectively, in MTX control compared to the neutral control. In comparison with the MTX control, the plant extract significantly prevented the decrease in catalase activity by 27.43% and 18.12% ($p < 0.001$) at the dose of 150 mg/kg, also by 32.23% ($p < 0.001$) and 18.12% ($p < 0.05$) at 250 mg/kg in liver and kidney respectively, by 30.03% ($p < 0.001$) in liver at 350 mg/kg. L-carnitin induced significant increase in catalase activity ($p < 0.001$) by 20.54% and in SOD by 21.08% ($p < 0.05$), in the liver of animals compared to the MTX control.

A significant increase in glutathione concentration was observed with plant extract by 66.67% and 69.91% ($p < 0.001$) at the dose of 250 mg/kg, by 70.59% and 71.50 % ($p < 0.001$) at 350 mg/kg and by 71.27% and 66.73% ($p < 0.001$) with L-carnitin in liver and kidney, respectively compared to MTX control.

Nitrites level significantly increased with plant extract by 21.68 % ($p < 0.001$) in kidney at the dose of 250 mg/kg, 17.35% and 21.97% ($p < 0.001$) in liver and kidney, respectively in L-carnitin control compared to MTX control.

In comparison to MTX control, total tissue proteins concentration increased in animals treated with plant extract by 45.67% and 38.46% ($p < 0.01$) at the dose 250 mg/kg, 48.33% and 56.65 % ($p < 0.01$) at 350 mg/kg and by 51.47 %, ($p < 0.01$) and 70.99 % ($p < 0.001$) with L-carnitin in liver and kidney, respectively.

The MDA level significantly decreased by 54.30%and 63.35 % ($p < 0.001$) at the extract dose of 150 mg/kg, 65.04% and 63.04 % ($p < 0.001$) at 250 mg/kg, by 93.37% and 61.48 % ($p < 0.001$) at 350 mg/kg and by 73.90% and 47.95 % ($p < 0.001$) with L-carnitin in the liver and kidney, respectively compared to MTX control. Compared to L-carnitin control, no significant change was observed in catalase, nitrites, SOD and MDA and plant extract whatever the dose. However, a significant decrease in glutathione level by 64.68% and 44.12 %, ($p < 0.001$) and in total proteins tissue by 32.78 %, ($p < 0.05$) and 52.87 %, ($p < 0.01$) was noted in liver and kidney, respectively with extract at 150 mg/kg.

Table 4. Effect of aqueous extract of *Sorindeia juglandifolia* on some oxidative stress parameters in liver and kidney tissues of animals.

	Catalase (µmol of H_2O_2/g of organ)	Glutathione (µmol/g of organ)	SOD (unit of SOD/g of organ)	Nitrites (µmol/g of organ)	Protein tissue (mg/dL)	MDA (µM/g of organ)
Liver						
Neutral control	3.17 ±0.10	12.85 ±0.37	10.28 ±0.11	659.99 ±29.48	0.81 ±0.02	1.46 ±0.19
MTX control	2.62 ±0.03	3.18 ±1.68$^{\neq\neq}$	8.13 ±0.12$^{\neq\neq}$	510.74 ±24.02$^{\neq\neq}$	0.39 ±0.02$^{\neq\neq}$	6.41 ±0.75$^{\neq\neq}$
L-carnitin	3.30 ±0.11$^{\alpha\alpha\alpha}$	11.10 ±0.91$^{\alpha\alpha\alpha}$	10.30 ±0.13$^{\alpha\alpha\alpha}$	617.98 ±2.18$^{\alpha\alpha}$	0.80 ±0.02$^{\alpha\alpha}$	1.67 ±0.20$^{\alpha\alpha\alpha}$
S.jug150 mg/kg	3.62 ±0.10$^{\alpha\alpha\alpha}$	3.92 ±0.17$^{\neq\neq\neq***}$	8.91 ±0.11	546.33 ±26.42	0.54 ±0.02$^{\neq*}$	2.92 ±0.21$^{\alpha\alpha\alpha}$
S.jug250 mg/kg	3.62 ±0.10$^{\alpha\alpha\alpha}$	3.92 ±0.17$^{\neq\neq\neq***}$	8.91 ±0.11	546.33 ±26.42	0.54 ±0.02$^{\neq*}$	2.92 ±0.21$^{\alpha\alpha\alpha}$
S.jug350 mg/kg	3.75 ±0.11$^{\alpha\alpha\alpha}$	10.84 ±0.62$^{\alpha\alpha\alpha}$	9.98 ±0.11	531.05 ±36.57	0.75 ±0.02$^{\alpha\alpha}$	0.42 ±0.04$^{\alpha\alpha\alpha}$
Kidney						
Neutral control	3.39 ±0.54	29.95 ±1.74	8.22 ±0.21	635.53 ±29.48	0.50 ±0.02	2.80 ±0.34
MTX control	2.46 ±0.02$^{\neq\neq}$	4.96 ±2.48$^{\neq\neq}$	6.79 ±0.13$^{\neq\neq}$	477.39 ±25.03$^{\neq\neq}$	0.14 ±0.02$^{\neq\neq}$	5.80 ±0.44$^{\neq\neq}$
L-carnitin	2.81 ±0.08	14.91 ±1.49$^{\neq\neq\alpha\alpha\alpha}$	8.01 ±0.22	611.81 ±13.10$^{\alpha\alpha\alpha}$	0.49 ±0.09 $^{\alpha\alpha\alpha}$	3.02 ±0.77$^{\alpha\alpha\alpha}$
S.jug150 mg/kg	3.00 ±0.09$^{\alpha}$	8.33 ±1.14$^{\neq\neq\neq***}$	7.26 ±0.13	562.39 ±2.18	0.23 ±0.02$^{\neq\neq*}$	2.12 ±0.45$^{\alpha\alpha\alpha}$
S.jug250 mg/kg	3.00 ±0.13$^{\alpha}$	16.38 ±1.66$^{\neq\neq\neq\alpha\alpha\alpha}$	7.70 ±0.11	609.58 ±24.02	0.46 ±0.14$^{\alpha\alpha**}$	2.14 ±0.41$^{\alpha\alpha\alpha}$
S.jug350 mg/kg	2.87 ±0.07	17.41 ±1.53$^{\neq\neq\neq\alpha\alpha\alpha}$	7.74 ±0.10	524.09 ±24.11	0.32 ±0.02$^{\neq}$	2.23 ±0.45$^{\alpha\alpha\alpha}$

Values are expressed as mean ± SEM (n= 5). Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration. $^{\neq}p < 0.05$, $^{\neq\neq}p < 0.01$, $^{\neq\neq\neq}p < 0.001$: significant difference compared to the neutral control. $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$, $^{\alpha\alpha\alpha}p < 0.001$: significant difference compared to methotrexate control. *p < 0.05, **p < 0.001: difference significant compared to L-carnitin control.

3.7. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on hematological parameters

The treatment with plant extract followed the methotrexate administration for 10 days do not significantly modify hematological parameters among experimental groups (Table 5).

Table 5. Effects of aqueous extract of *Sorindeia juglandifolia* leaves on hematological parameters of animals treated with methotrexate

Blood parameters	Neutral control	MTXcontrol	L-carnitin control	S.jug150 mg/kg	S.jug250 mg/kg	S.jug350 mg/kg
RBC (10^6/µL)	9.92 ±0.20	13.10 ±0.91	11.88 ±0.48	11.06 ±0.19	11.99 ±0.52	11.22 ±0.25
Hemoglobin(g/dL)	9.70 ±1.60	10.14 ±1.64	9.00 ±1.23	8.20 ±0.95	10.64 ±1.82	9.50 ±1.41
Hematocrit (%)	27.84 ±0.40	35.50 ±2.29	35.44 ±2.28	33.84 ±1.71	37.71 ±3.17	33.80 ±1.69
Platelets (10^3/µL)	359.50 ±21.38	345.50 ±16.44	334.60 ±12.51	297.00 ±0.70	313.000 ±4.94	300.60 ±0.50

WBC (10^3/µL)	8.54±0.01	6.16±0.36	8.52±0.03	8.44±0.05	9.20±0.02	8.54±0.01
Lymphocyte (%)	71.78±1.02	73.10±2.05	79.18±1.73	78.58±2.15	73.34±2.19	77.52±1.32
Monocyte (%)	9.42±0.79	9.18±0.45	7.12±0.26	8.58±0.29	10.00±0.55	11.70±0.14
Granulocyte (%)	11.82±0.62	10.32±0.15	6.48±0.53	7.12±0.28	9.62±0.22	7.13±0.21

Values are expressed as mean ± SEM (N = 5). Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration. ###p < 0.001: significant difference compared to the neutral control.

3.8. Effects of *Sorindeia juglandifolia* leaves extract on the liver and kidney tissues in methotrexate-treated animals

3.8.1. Effects of *Sorindeia juglandifolia* leaves extract on the liver tissues

Figure 2 shows the effects of plant extract on liver of methotrexate-treated animals. The histology of the normal liver (Figure 2A) presents normal architecture with hepatocyte (H), portal vein (PV), bile canaliculi (BC) and hepatic artery (HA). Liver damage induced by methotrexate resulted in periportal fibrosis (PPF) and portal fibrosis (PF) (Figure 2B). Liver architecture of animal treated with plant extract whatever the dose (Figures 2C, 2D and 2E) or with L-carnitin (Figure 2F) was similar to that of the neutral control, though the presence of sinusoidal dilatation (SD) observed in the parenchyma of animals treated with extract (350 mg/kg) and L-carnitin.

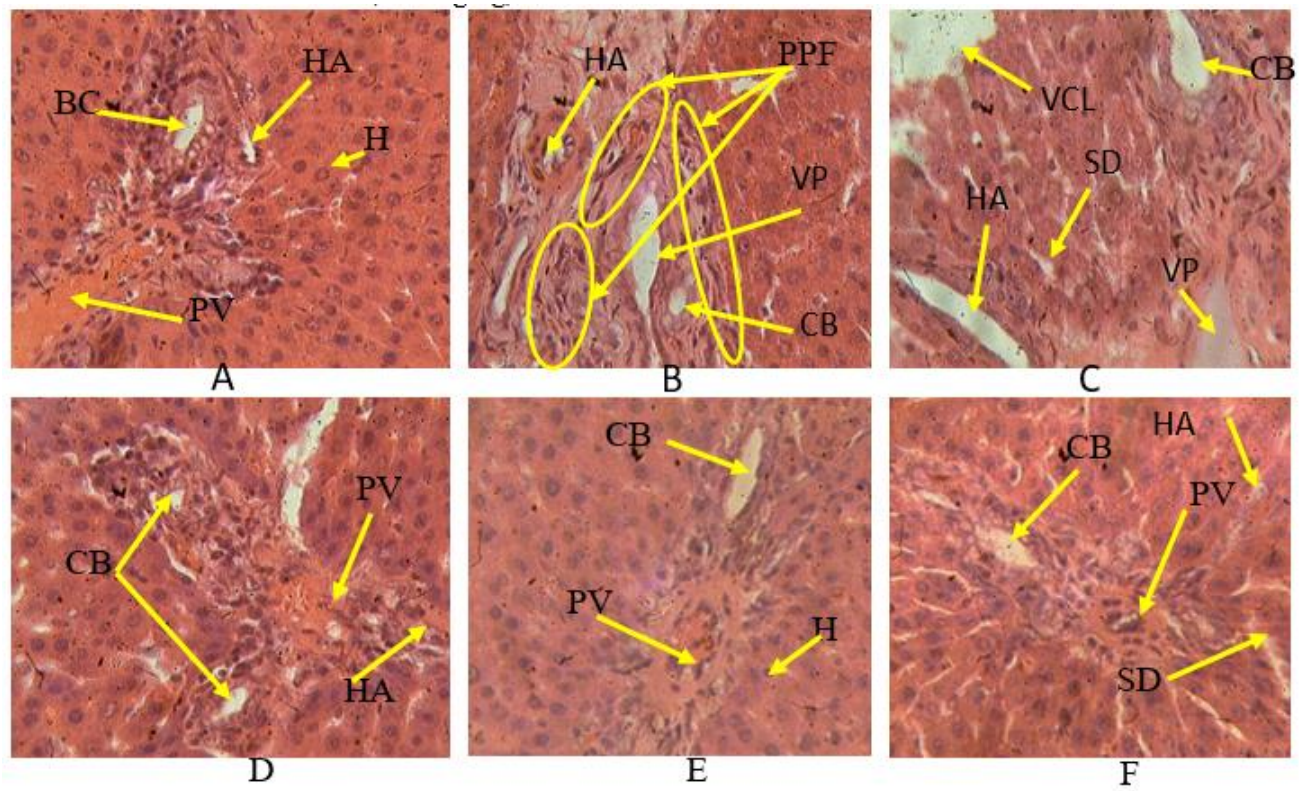

Figure 2. Effects of *Sorindeia juglandifolia* leaves extraction on histological change in the liver tissue in neutral control (A), Methotrexate control (B), L-carnitin control (C), S.jug 150 mg/kg (D), S. jug 250 mg/kg (E), S. jug 350 mg/kg (F) (Hematoxylin-Eosine ×400).

Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia*at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration. H=hepatocyte, PV = portal vein, CB = bile canaliculi, HA=hepatic artery, PPF=periportal fibrosis, PF=portal fibrosis, SD = sinusoidal dilatation.

3.8.2. Effects of *Sorindeia juglandifolia* leaves extract on the kidney tissue

The effects of *S. juglandifolia* extract on the histology of kidney tissue of animal treated with methotrexate are shown in the Figure 3. The kidney tissue of neutral control presents a normal architecture in kidney with proximal tube (PT), distal tube (DT), glomerular (G) and urine space (US) (Figure 3A). MTX induced some disorganization in kidney parenchyma, with glomerular fibrosis (GF), periglomerular fibrosis (PGF) and necrosis focus (Ne) in MTX control (Fig 3B). The plant extract administration prior methotrexate protected from damage showing normal architecture of kidney (Figure 3D, E and F) closed to the neutral control. Histology of kidney tissue of animals treated with L-carnitin showed normal architecture.

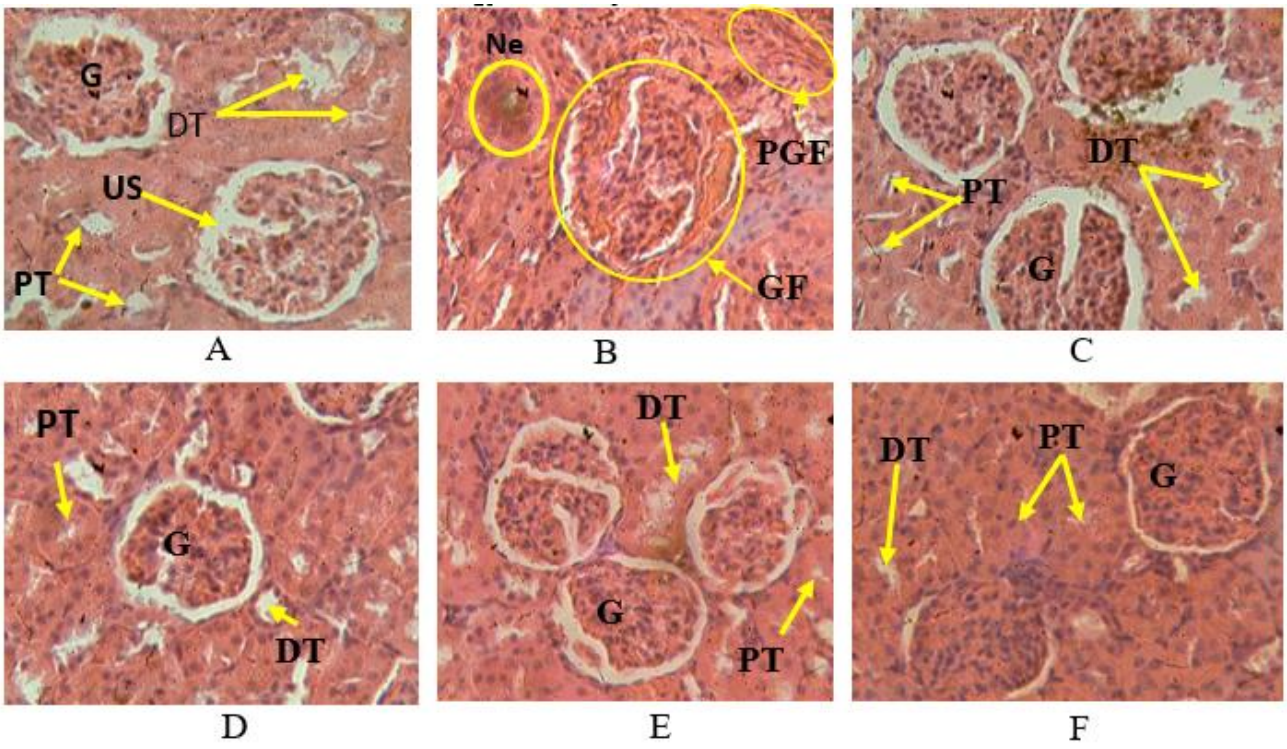

Figure 3. Effects of *Sorindeiajuglandifolia* leaves extraction on histological change in the kidney tissuein neutral control (A), Methotrexate control (B), L-carnitin control(C), S.jug 150 mg/kg (D), S. jug 250 mg/kg (E), S. jug 350 mg/kg (F)(Hematoxylin-Eosine ×400).

Neutral control = Healthy animals receiving distilled water, MTX control = animals receiving distilled water and methotrexate 20 mg/kg; L-carnitin control = animals receiving L-carnitin (500 mg/kg) and methotrexate (20 mg/kg), S.jug. = animal treated with *Sorindeia juglandifolia* at 150 mg/kg (S.jug 150 mg/kg), 250 mg/kg (S. jug 250 mg/kg) and 350 mg/kg (S.jug 350 mg/kg) followed to methotrexate administration.GF = glomerular fibrosis, PGF = periglomerular fibrosis, PT = proximal tube, DT = distal tube, G = glomerular, US = urine space, Ne = necrosis.

4. Discussion

The study aimed to investigate the effects of aqueous extract of *Sorindeia juglandifolia* leaves on methotrexate-induced liver and kidney damage. The methotrexate administration for ten days to healthy animal led to a decrease in body weight and an increase of relative weight of liver whereas that of kidney weight has decreased. These changes in MTX administration have been described as methotrexate side-effects with a high-dose therapy, resulting from the inhibition of dihydrofolate reductase (DHFR), an enzyme that implicates in the tetrahydrofolate synthesis and therefore, a prevention of synthesis of DNA, RNA, thymidylates, and proteins inducing a hypoproteinemia.[23,24] Likewise, methotrexate affects muscle mass and quality causing muscle wasting, which in turn could cause alterations in energy balance and the depletion of adipogenic precursors [25], justifying the loss of weight observed in the present study.

The relative weight increase of liver might be due to the proliferation of collagen fibers that cause fibrosis in the parenchyma as observed in liver tissue of MTX control group. The consecutive administration of plant extract has prevented these

changes in animals, indicating the protective effects of plant. The plant activities could be due to the presence of phenol compounds as flavonoid, terpenoid that able to inhibit toxin effects and prevent the liver and kidneys impairment [26]. Besides, flavonoids component capture metallic ion involved in free radical's production [27], inhibit enzymes activity involved in the production of cyclooxygenases, lipoxygenases and arachidonic acid, thereby inactive P-glycoprotein involved in the resistance of phenotypic cancer cells that can be issue to genetic mutation by drug [28].

The hypoproteinemia, the increase in serum alkaline phosphatase (ALP) level, total bilirubin level and transaminases (ALAT and ASAT) activities observed after MTX administration are conventional signs of liver failure. Indeed, methotrexate is an acid folic antagonist that blocks the synthesis of purines and pyrimidines by inhibiting of several key enzymes, resulting to its toxic effects that include liver damage by adenosine receptor pathway [17]. Methotrexate generates radical oxygen species that attack cells and damage their functions (membrane fluidity and membrane bound enzyme) as expressed in our study by the increase in MDA (a marker of lipid peroxidation); that lead to the leakage of these enzymes into the blood stream [29,30]. The preventive effect of plant extract to liver dysfunction could be assigned to phenol compound like tannins, capable to trap free radical agents and therefore protect hepatocytes from damage [31].

The hypercholesterolemia, hypertriglyceridemia, the increase of the atherogen index and the decrease of HDL cholesterol observed after methotrexate administration for ten days sign of the occurrence of atherosclerosis development and coronary heart disease. This study is in concordance with those of Sabah and Yasmin [32] who reported that methotrexate induced an acid folic deficiency accompanied by increased risk of cardiovascular disease confirmed in the present study by lipid profile impairment. This dyslipidemia observed in the study also indicates liver injury and result in an imbalance between different types of cholesterol. Moreover, LDL cholesterol are more activated than HDL cholesterol because of the excessive lipolysis stimulation that generate fatty acids as alternative source of energy, that results in the fall of fatty acid stock into the liver [33]. This change was also observed into the liver tissue expressed by disorganization in the parenchyma. *Sorindeia juglandifolia* plant extract significantly reduced serum cholesterol, LDL-cholesterol and triglycerides and enhanced HDL-cholesterol, suggesting its blood modulation properties in dyslipidemia and/ or it can prevent acid folic deficiency. The ability regulatory of plant extract could be due to the presence of flavonoid acting by increasing of cholesterol HDL and by reducing of total cholesterol [34]. These polyphenols act by inhibition of hyperlipidemia either by decrease in pancreatic lipase activity, inhibit triglyceride hydrolysis [35] or modify lipid metabolism that result in their excretion into stool [36].

The administration of methotrexate at the dose of 20 mg/kg provoked kidney damage characterized by an increase in serum urea and creatinine. It has been shown that, the increase in this parameter in serum indicate an alteration in glomerular filtration [37], as showed in this study. This alteration is also confirmed by the micro-architectural disorganization of the kidney. All of these physiological changes reflect a kidney failure, known as methotrexate toxic effects resulting from an obstructive nephropathy [22] with pre-glomerular vascular resistance disturbance [38], direct glomerular toxicity [39] or tubes toxicity [40]. These changes damage homeostasis control of calcium with the active vitamin D production [41] by acting on tubular calcic epithelial channel and on the edge brush of intestinal cells during calcium reabsorption [42]. Dysnatremia observed in the study is a sign of liver and kidney failure. The hepatic metabolism of methotrexate could cause fibrosis and progressive cirrhosis [43]. Fibrosis induces resistance of portal vein and cause its vasodilatation that result in blood retention in liver portal system. Vasodilatation due to nitrites [44] and prostaglandin [45] induce infiltration of water in abdomen and therefore, a decrease of blood stream in juxtaglomerular engine leading to the renin synthesis which stimulate angiotensin II production and emphasize vasoconstriction system in kidney. This change induces a decrease of capillary filtration surface and therefore a decrease of glomerular filtration [46] as observed in the study. Indeed, the systemic vasodilatation lead to a liver dysfunction involving carotid baroreceptors stimulation, which activates anti-natriuretic system, especially the secretion of renin and angiotensin and stimulates the aldosterone hormone (ADH) synthesis, which induce water retention following by fibrosis or cirrhosis formation [47]. The plant extract has prevented kidney failure through the presence of flavonoid, tannins and coumarin components that act by preventing kidney abnormalities, suggesting the helpful activities of plant extract in the modulation of kidney function [48,49]**.** Flavonoids are known for their sodium homeostasis activing by Na+-K+-2Cl- cotransporter-1 (NKCC1) in epithelia's cells which result into the Na+ reabsorption via the epithelial Na+ channel (ENaC) or via the epithelial Na+ channel (ENaC) inhibitory leading to the reduction of Na+ reabsorption and decrease of volume-dependent elevated blood pressure [50]. They also modulate

calcium homeostasis by acting on reticulum endoplasmic calcium-ATPase, responsible to the transport of calcium out or into the cells [51].

It was observed a significant decrease in antioxidant defenses (SOD, nitric oxide (NO), glutathione, and catalase) in the liver and kidney of rats treated with methotrexate. These factors are implicated into the degradation of free radical's species [52,53]. In fact, methotrexate treatment generates some reactive oxygen (ROS) and nitrogen (RNS) species, inhibits cytosol NADP-dependent dehydrogenase and NADP malic enzyme, which reduce the level of glutathione, superoxide dismutase, catalase and ultimately reducing the effectivity of the antioxidant defence system [54].

The prevention of antioxidant defence system decrease by plant extract suggesting its regulatory effects on oxidative stress. This antioxidant property could be targeted by flavonoids, coumarin, triterpene, tannins containing into the extract that induce cell protection and detoxification effects [30,55]. Tannins trap free radical agents and therefore protect cells from cell damage [30]. They are also protons donors to free radicals produced in lipid peroxidation to stop chain reaction of lipid oxidation [31]. Coumarin and its derivatives represent one of the most active classes of compound possessing a wide spectrum of biological role as anti-inflammatory activities [56]. Unsaturated terpen as sterols has vital role in cells structural integrity of organism by controlling ions permeability [57]. Polyphenol could act either by fixing electron to give stable radicals and thereby inhibits, decelerates or blocks oxidation chain reaction in cells [58].

5. Conclusion

The present study demonstrated the preventive effect of aqueous extract leaves of S*orindeia juglandifolia* on methotrexate-induced liver and kidneys affections on *Wistar* rats. The plant extract has protected from liver damage by preventing the increase of transaminases activities alkaline phosphatase, total bilirubin, hypercholesterolemia and hypertriglyceridemia. Plant has also prevented to the kidney function impairment by modulating of creatinine, urea, protein and ions levels in serum and urine sample. The aqueous extract of S*orindeia juglandifolia* leaves protected from metabolic disorders, oxidative stress and architectural disorganization observed into the organs (liver and kidneys). These results confirm the hepato-nephroprotective effects of *Sorindeia juglandifolia* and could justify its traditional use.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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